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GENETIC VARIATION AMONG COTTON GERMPLASM FOR WATER-USE EFFICIENCY*

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QUISENBERRY J. E. and MCMICHAEL B. L. *Genetic variation among cotton germplasm for water-use efficiency*. ENVIRONMENTAL AND EXPERIMENTAL BOTANY **31,** 453–460, 1991.—Cotton (*Gossypium* spp.) genotypes including three species, five modern cultivars, one strain and 18 primitive race stocks were grown in two greenhouse experiments to determine differences in water-use efficiency (WUE). Water-use efficiency was defined as "the weight of total biomass (shoot and root) produced per unit of water transpired". Plastic containers were filled with air-dried soil, and water was added until the soil reached field capacity. Plants were then grown (without further watering) until reaching the permanent wilting point. Data on plant biomass (shoot and root), water use, and days to permanent wilting were then collected. Differences occurred between the two experiments in water used prior to permanent wilting. No significant differences between genotypes were observed in the amount of water used, but differences did occur in biomass production and days to permanent wilting. Genotypes did not interact with environments for water used, biomass produced, or WUE. Differences did occur among genotypes between experiments for WUE. Genotypic means showed a 28% range in WUE between the highest vs the lowest entries and a 14% improvement above the best cultivar. *G. herbaceum* L. and *G. barbadense* L. fell within the distribution range for *G. hirsutum* L. Primitive race stocks of *G. hirsutum* were more efficient as a group in water use than were modern cultivars of the species.

INTRODUCTION

WATER-USE efficiency (WUE) defined here as the amount of total biomass produced on a dry weight basis per unit water used (transpired) by the plant is one of the most desirable and most elusive traits in plant breeding. Although useful in any climate, a high efficiency for this trait would certainly be of value in areas with inadequate rainfall and/or available irrigation. Over time, plant breeders have improved the yield of crop plants/unit of water used by increasing the harvest index (i.e. the relative proportion of total biomass harvested as yield). Selection for harvest index has been in practice directly or indirectly since crop plants were first domesticated, and continues today.

TANNER and SINCLAIR[10] reviewed the search for genetic differences in WUE and concluded that interspecific differences do occur, but that intraspecific differences were small. Later research by FARQUHAR and RICHARDS,[4] using ^{12}C–^{13}C discrimination analyses, demonstrated that small, but important differences can occur within a species. In cotton (*Gossypium hirsutum* L.), comparisons of biomass produced under field conditions of water deficiencies have demonstrated significant differences among the genotypes that were tested,[8] but because of the difficulties involved in measuring soil water and root mass,

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conclusions on WUE were not drawn. QUISENBERRY *et al.*[9] compared the WUE of two primitive race stocks of cotton, T25 and T169, grown in containers in the greenhouse and demonstrated small, but significant, differences between them.

Over the past 15 years, we have grown a large portion of the world collection of *Gossypium* spp. under field conditions with declining soil water. The general response of the germplasm has been the same, i.e. as soil water declined, growth also declined. In most cases, it appeared that the advantage of a particular genotype in terms of productivity was that more biomass was produced prior to the onset of the severe water deficit. Some genotypes grow faster than others under optimal water conditions and a relationship exists between the amount of biomass produced under optimal water conditions vs under declining water. Occasionally, a genotype will grow for a longer time, although at a slower growth rate, under a declining water supply. That genotype may ultimately achieve more biomass than will others. Such observations have been made where competition between genotypes was minimal.

Water stress can impact the partitioning of the total biomass between roots and shoots.[6] EATON[3] demonstrated that prolonged periods of stress reduced the shoot biomass while AUNG[2] reported an increase in root biomass with increasing water deficits. Root length has also been shown to increase under stress conditions.[5] In many studies, the ability of the plant to adjust osmotically has been shown to impact the stress response by allowing plant functions to continue at lower plant water potentials than would normally occur.[1,7] The authors know of no reports, however, that directly link the ability of the plant to osmoregulate to increases in water-use efficiency.

Since water stress can influence the partitioning of the total biomass produced and since it is extremely difficult to determine total biomass (roots as well as shoots) under field conditions, this research was initiated to estimate differences in WUE among an array of cotton germplasm grown under different environmental conditions in greenhouse experiments. The results should provide information required for selection of superior genotypes for further evaluation under field conditions.

MATERIALS AND METHODS

Twenty-five cotton genotypes were chosen that, based on past observations, showed various responses to declining soil water. Eighteen were from the world collection of primitive (or "dooryard") race stocks of *G. hirsutum*; one was a *G. herbaceum* (designation and origin unknown), another was a modern *G. barbadense* cultivar ("Pima S-5"); four were modern *G. hirsutum* cultivars ("Paymaster 145", "Coker 5110", "Tamcot CAMD-E", and "Deltapine 61") and the last was an early fruiting, fast maturing experimental *G. hirsutum* strain from our breeding program (Lubbock Dwarf). Of the 18 primitive race stocks, six were race latifolium (T50, T80, T151, T169, T185, and T252), four were race punctatum (T15, T25, T45, and T115), two race morrilli (T171 and T283), two race marie-galante (T141 and T184), two race richmondi (T461 and T256), one race palmeri (T1), and one race yucatanense (T1236). Although the race designation is not a true taxonomic category, it does describe a general group of phenotypic characters common among the various entries in that group. All primitive race stocks used are photoperiodic and require long nights to flower and set seed. Therefore, under summer field conditions at Lubbock, TX the stocks are completely vegetative; if grown under greenhouse conditions in the winter they will reproduce.

In the experiments conducted in the greenhouse, water-use efficiency was defined as "the total biomass (shoot and root) produced per unit of water transpired". Plastic pots (volume 37.8 l, mass 1 kg) were filled with 56.4 kg of air-dried Amarillo loam soil (fine-loamy, mixed, thermic Aridic Paleustalf), and enough water was added and allowed to drain until the termination of drainage from the small holes in the bottom of the pots. When drainage ceased, the surface of each pot was covered with plastic to prevent evaporation and the holes in the bottom of the pots were sealed with tape to reduce additional water loss. All pots were weighed 3 days later to establish the initial weight for determination of soil water use.

Experiments were duplicated in two environments in the greenhouse. The first experiment was planted on 24 October 1984, while the second

Table 1. Means for environmental parameters for greenhouse experiment 1 (no fans) and experiment 2 (with fans)

Parameter	Experiment 1	Experiment 2
Air temperature (°C)		
Average daily max.	30.6 ± 2.3	33.9 ± 3.3
Average daily min.	25.4 ± 1.1	25.2 ± 1.8
Relative humidity (%)		
Average daily max.	58.9 ± 9.8	62.3 ± 11.7
Average daily min.	40.8 ± 7.5	37.8 ± 8.6
Windspeed (m/sec)		
Average daily max.	0.2 ± 0.05	4.0 ± 1.2*
Average daily min.	0.1 ± 0.06	0.2 ± 0.07
Solar radiation (MJ m^{-2} hr^{-1})		
Mid-day outdoors	2.95 ± 0.23	3.15 ± 0.16
Mid-day in greenhouse	2.60 ± 0.10	2.85 ± 0.11

*Fans were operated from 0800 to 2000 hr each day in this experiment.

was planted on 28 January 1985. Five pots were used to grow each entry in each experiment in a randomized complete block design. Five seed of each entry were planted per pot. Fourteen days after planting, a small cut was made in the plastic surface to allow hypocotyls and cotyledons to emerge above the barrier. Each pot was then thinned to one plant, and tape was placed around the hypocotyl to prevent evaporation from the opening in the plastic sheet. Plants were observed daily, and any dropped leaves were saved for inclusion in the total biomass (dry wt) produced by the plant. The plants were grown until the third true leaf from the top of the plant failed to recover visible turgor at predawn. This was defined as the "permanent wilting point" and the date that this occurred was recorded for each pot. The top of the plant was then harvested, dried at 80°C along with any leaves that were shed during the growth period, and weighed. The pot was then weighed to determine the amount of water used. After the pot was weighed, the roots were washed from the soil, dried at 80°C, and weighed. During the course of these experiments, greenhouse maximum and minimum temperatures and relative humidities were recorded at a height of 25 cm above the canopy. The means and ranges for the environmental parameters measured are shown in Table 1. Thermostats were set at 25°C, and night temperatures did not go significantly below that temperature. Although attempts were made to control day-time temperatures to 25°C, air temperature often exceeded 25°C by approximately 5–7°C. Radiation transmission within the greenhouse was greater than 90% of that measured outdoors. During the first experiment, air movement within the greenhouse was minimal and leaf boundary resistance was quite high. During the second experiment, large oscillating fans moved air at the canopy level at approximately 4 m/sec thereby greatly reducing the leaf boundary layer resistance. The fans operated each day from 0800 to 2000 hr.

RESULTS AND DISCUSSION

The two experiments differed in water used and days to permanent wilting (Tables 2, 3, 5), but not in biomass produced. Because the data were collected when the plants reached permanent wilting, these results suggest that the air movement provided by the fans in experiment 2 caused such wilting to take place sooner than in experiment 1 (with no fans). The effect occurred even though the fans were not used at night and the plants were in a relatively humid greenhouse for 12 hr prior to the determination of permanent wilting.

The genotypes differed in biomass produced and days to permanent wilting (Tables 4, 5). The methodology of the experiments dictated that the genotypes did not differ in the water available to them; however, genotypes may have differed in the level of soil water remaining at the time when permanent wilting occurred. In fact, all genotypes wilted after using the same amount of water. Differences among genotypes in days to permanent wilting suggested that some genotypes produced biomass slower than did others, which initially appeared to be confirmed by genotypic differences in biomass produced. However, linear correlation coefficients between biomass produced and days to permanent wilting were not statistically significant (experiment 1, $r^2 = 0.03$; experiment 2, $r^2 = 0.01$).

Genotype by experiment interactions were not significant for water used or biomass produced, but were significant for days to permanent wilting (Table 5). The lack of a significant interaction for water used further supports the hypothesis that differences in that trait between experiments 1 and 2 were solely a result of environmental differences, namely the influence of windspeed on the reduction of the days to permanent wilting.

Table 2. Means for total biomass, water use, water-use efficiency, and days to permanent wilting for 25 cotton genotypes grown in the greenhouse (experiment 1)

Genotype	Total biomass (g)	Water use (kg)	Water-use efficiency (g/kg)	Days to permanent wilting
T80	20.31	9.480	2.14	75.2
T256	20.44	9.775	2.09	73.6
T283	20.24	9.667	2.09	68.3
T461	20.00	9.684	2.06	71.0
T1236	18.28	9.911	1.85	83.8
G. herbaceum L.	20.19	9.677	2.08	74.3
T171	19.93	9.696	2.06	74.2
T15	20.86	9.775	2.12	70.2
T184	17.57	9.458	1.85	83.0
T252	20.23	9.696	2.08	71.3
T1	18.76	9.526	1.97	71.7
T25	19.33	9.616	2.01	70.4
T45	18.96	9.616	1.96	70.8
T141	16.70	9.639	1.74	78.0
T115	16.69	9.775	1.71	82.8
Coker 5110	19.36	9.730	1.99	67.4
T151	17.88	9.458	1.89	67.4
T50	18.93	9.390	2.02	63.0
G. barbadense L.				
"Pima S-5"	18.00	9.458	1.92	70.2
Paymaster 145	18.17	9.594	1.87	70.0
T185	17.40	9.606	1.81	70.3
Tamcot CAMD-E	16.45	9.129	1.80	70.3
T169	16.32	9.412	1.76	70.6
Lubbock Dwarf	15.84	9.798	1.62	76.2
Deltapine 61	16.90	9.548	1.76	64.8
Means	18.55	9.605	1.93	72.3
LSD (0.05)	1.01	0.20	0.04	3.00

The non-significant interaction for biomass produced demonstrated that genotypes with a large biomass produced in experiment 1 also produced large biomass in experiment 2. The significant interaction for days to permanent wilting was due to changes in magnitude, not changes in growth rate. A linear correlation coefficient between experiments 1 and 2 for days to permanent wilting of the different genotypes was 0.08 ($P < 0.01$) showing that those genotypes that took longer to reach permanent wilting in experiment 1 tended to do the same in experiment 2.

The experiments significantly impacted WUE (Table 6). Experiment 1 had a significantly lower WUE than did experiment 2 (Tables 2, 3). An important factor that could contribute to these differences is the fact that an extended time was required in experiment 1 (i.e. 72.3 days) to reach permanent wilting as compared to experiment 2 (60.7 days) (Table 5) due to the effects of the fans reducing the boundary layer. Plants in experiment 1 may have achieved their maximal dry weights near the same time, relative to one another, as did plants in experiment 2. However,

Table 3. *Means for total biomass, water use, water-use efficiency, and days to permanent wilting for* 25 *cotton genotypes grown in the greenhouse* (*experiment* 2)

Genotype	Total biomass (g)	Water use (kg)	Water-use efficiency (g/kg)	Days to permanent wilting
T80	19.45	8.505	2.29	60.0
T256	19.72	8.550	2.30	61.2
T283	19.74	8.460	2.33	63.2
T461	20.66	8.664	2.39	62.8
T1236	17.13	8.505	2.01	69.2
G. herbaceum L.	19.64	8.369	2.35	58.2
T171	18.84	8.573	2.20	62.4
T15	19.84	8.346	2.37	58.4
T184	16.37	8.210	2.00	67.4
T252	18.97	8.437	2.24	60.0
T1	19.24	8.823	2.18	61.0
T25	17.84	8.505	2.10	61.6
T45	18.82	8.686	2.17	58.2
T141	16.89	8.528	1.98	65.0
T115	16.12	8.392	1.92	62.4
Coker 5110	17.86	8.346	2.14	59.0
T151	18.26	8.392	2.18	57.8
T50	18.34	8.392	2.19	56.8
G. barbadense L.				
"Pima S-5"	16.98	8.437	2.01	58.4
Paymaster 145	16.71	8.193	2.09	58.0
T185	16.67	8.392	1.99	59.0
Tamcot CAMD-E	16.70	8.233	2.03	58.2
T169	16.67	8.301	2.00	58.8
Lubbock Dwarf	15.97	8.420	1.90	61.5
Deltapine 61	16.71	8.414	1.98	58.0
Means	18.00	8.443	2.13	60.7
LSD (0.05)	0.99	0.21	0.08	2.50

because the plants in experiment 1 may not have been under the stress associated with constant daily air movement, they may have continued to recover turgor overnight and to use water and fix carbon for at least a short period of time each morning. If osmoregulation were occurring in these plants, as a result of the short duration stress, then the stomates may have remained open for a longer period of time during the morning hours as has been reported in other instances.[1] Following this brief period each morning, water use could have continued but respiration may have equaled or exceeded photosynthesis with no significant amounts of additional dry matter accumulated, resulting in the significant differences between the experiments. Measurements of photosynthesis, turgor, or respiration were not made in these studies.

There were significant differences in WUE between the genotypes over both experiments. Mean values (averaged across both experiments) for each genotype for WUE are shown in Table 4. The range in WUE was 0.49 g biomass/kg of water used. The genotype with the highest WUE was T15 which was 28% more efficient in water use than was the lowest entry, Lubbock Dwarf. Except for the *G. herbaceum* geonotype, all genotypes with higher WUE were from the primitive

Table 4. Means for 25 cotton genotypes averaged over two greenhouse experiments for total biomass, water use, water-use efficiency, and days to permanent wilting

Genotype	Total biomass (g)	Water use (kg)	Water-use efficiency (g/kg)	Days to permanent wilting
T80	19.88	9.00	2.22	67.6
T256	20.08	9.17	2.20	67.4
T283	19.99	9.07	2.21	65.8
T461	20.33	9.17	2.23	66.9
T1236	17.71	9.21	1.93	76.5
G. herbaceum L.	19.52	9.03	2.22	66.3
T171	19.39	9.14	2.13	68.3
T15	20.35	9.07	2.25	64.3
T184	16.97	8.84	1.93	75.2
T252	19.60	9.07	2.16	65.7
T1	19.00	9.18	2.08	66.4
T25	18.59	9.07	2.06	66.0
T45	18.89	9.16	2.07	64.5
T141	16.80	9.10	1.86	71.5
T115	16.41	9.10	1.82	72.6
Coker 5110	18.61	9.04	2.07	63.2
T151	18.07	8.93	2.04	62.6
T50	18.64	8.89	2.11	59.9
G. barbadense L.				
"Pima S-5"	17.49	8.95	1.97	64.3
Paymaster 145	17.44	8.89	1.98	64.0
T185	17.04	9.01	1.90	64.7
Tamcot CAMD-E	16.58	8.68	1.92	64.3
T169	16.50	8.86	1.88	64.7
Lubbock Dwarf	15.91	9.11	1.76	68.9
Deltapine 61	16.81	8.98	1.87	61.4
LSD (0.05)	0.79	0.20	0.08	2.08

Table 5. Analyses of variance over experiments and means within each experiment for water used, biomass produced, and days to permanent wilting for 25 cotton entries grown in two experiments in a greenhouse

Source of variation	df	Mean squares: Water used	Biomass produced	Permanent wilting
Experiments (Exp.)	1	83.09*	17.53	8514.7*
Reps/Exp.	8	0.24	21.82	37.0
Genotypes (G)	24	0.18	20.26*	158.0*
G × Exp.	24	0.10	1.15	29.0*
Error	192	0.10	1.57	10.8
		Means		
		kg	g	days
Experiment 1		9.60 a†	18.5 a	72.3 a
Experiment 2		8.45 b	18.0 a	60.7 b

*Statistically significant at the 0.01 probability level.
† Means followed by the same letter are not significantly different at the 0.05 probability level based on an LSD test.

race stocks of *G. hirsutum*. In general, current cultivars were lower in WUE than were the primitive race stocks. Comparisons among the seven races of *G. hirsutum* did not show any race to be more efficient than the others. The *G. herbaceum* and *G. barbadense* entries fell within the normal distribution of the *G. hirsutum* germplasm.

Table 6. Analyses of variance over experiments and means within each experiment for water-use efficiency (WUE) for 25 cotton entries grown in two experiments in a greenhouse

Source	df	Mean squares WUE
Experiments (Exp.)	1	2.48*
Reps/Exp.	8	0.19
Genotypes (G)	24	0.21*
G × Exp.	24	0.01
Error	192	0.02
		Means
		g/kg
Experiment 1		1.93 b†
Experiment 2		2.13 a

*Statistically significant at the 0.01 probability level.
† Means followed by the same letter are not significantly different at the 0.05 probability level based on an LSD test.

In conclusion, we have demonstrated that intraspecific variation for WUE exists among cotton germplasm. If the variation described can be translated to field plots, lint yields may be increased when water is the limiting factor. The highest modern cultivar of those tested, Coker 5110, was 14% less efficient in the use of water than was the highest race stock, T80. These results indicate that potential for improvement exists in the cotton germplasm for more efficient water use but further evaluations are necessary before improved agronomic types can be developed.

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